

Cold vapour atomic fluorescence determination of mercury in milk by slurry sampling using multicommutation

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Abstract

A highly sensitive mechanized method has been developed for the determination of mercury in milk by atomic fluorescence spectrometry (AFS). Samples were sonicated for 10 min in an ultrasound water bath in the presence of 8% (v/v) aqua regia, 2% (v/v) antifoam A and 1% (m/v) hydroxylamine hydrochloride, and after that, they were treated with 8 mmol l^{-1} KBr and 1.6 mmol l^{-1} KBrO_3 in an hydrochloric medium. Atomic fluorescence measurements were made by multicommutation, which provides a fast alternative in quality control analysis, due to the easy treatment of a large number of samples (approximately 70 h^{-1}), and is an environmentally friendly procedure, which involves a waste generation of only 94.5 ml h^{-1} as compared with the 605 ml h^{-1} obtained by using continuous AFS measurements. The limit of detection found was 0.011 ng g^{-1} Hg in the original sample. The method provided a relative standard deviation of 3.4% for five independent analysis of a sample containing 0.30 ng g^{-1} Hg. To validate the accuracy of the method, a certified reference material NIST-1459 (non-fat milk powder) containing $0.3 \pm 0.2 \text{ ng g}^{-1}$ Hg was analysed and a value of $0.27 \pm 0.06 \text{ ng g}^{-1}$ Hg was found. A comparison made between data found by the developed procedure and those obtained by microwave-assisted digestion and continuous AFS measurements evidenced a good comparability between these two strategies. Results obtained for commercially available milk samples varied between 0.09 and 0.61 ng g^{-1} Hg depending on the type of sample and its origin. The confluence of the analytical waste with a 6 mol l^{-1} NaOH allowed us to reduce the waste generation in a working session from 11 to 5 g solid residue with a matrix of $\text{Fe}(\text{OH})_3$ which contributes to the deactivation of traces of heavy metals presents in the samples that does not form volatile hydrides.

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1. Introduction

Mercury is a toxic element that occurs naturally at trace levels in the environment by natural degassing of the earth's crust, being the human activities the most important source of mercury contamination with emissions of the order of 6000 t per year. All chemical forms of mercury could cross the placental barrier and also are secreted to milk and, because of that, it is important to control the presence of Hg in milk samples [1].

To date there are only few studies on mercury concentration in milk, the main part of which were focussed on human milk contamination. It has been reported concentrations

from <0.2 to $6.86 \mu\text{g l}^{-1}$ [2] and from <0.25 to $11.7 \mu\text{g l}^{-1}$ [3] in human milk. High amounts of mercury in human milk were attributed to both, the presence of amalgam fillings in mother's teeth and fish consumption. Average concentration of Hg in human milk found at the Faroe Islands was $2.45 \mu\text{g l}^{-1}$ [4], where the typical diet includes whale meat and blubber, and only $0.6 \pm 0.4 \text{ ng g}^{-1}$ in Sweden [5].

However, from our knowledge, there are only one reference to the concentration of Hg in infant formula, being reported data from 0.4 to $2.5 \mu\text{g l}^{-1}$ [2] and no studies were found on the presence of mercury in commercially available cow milk. So, one of the aims of this work has been to provide data on Hg content in milk samples available in the Spanish market.

Today research, involving environmental science, agriculture, industrial processes, etc., presents a high demand

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for analytical results. To attend to those demands it is necessary to improve analytical procedures through automation in order to obtain a high throughput capability. In this sense, multicommutation, a mechanisation strategy based on the use of three-way solenoid valves for reagent's insertion in a flow network, provides an enhancement of the laboratory productivity [6] and additionally it offers the possibility to drastically reduce the complexity of flow manifolds required for determinations involving the use of several reagents, and reduces also the reagent consumption and the total amount of waste generated through the reduction of the use of continuously pumped channels [7,8]. So, multicommutation could be considered as an environmentally friendly analytical alternative to minimize mercury waste generation in practical analysis [9,10].

In this paper, a flow system integrated by computer-controlled discrete commutation device is proposed for the determination of mercury in milk samples by cold vapour atomic fluorescence spectroscopy (CV-AFS). The analytical performance of the method and practical parameters, such as laboratory productivity, reagents consumption and waste generation, have been evaluated. On the other hand, liquid toxic wastes were deactivated and minimized through an on-line treatment based on coprecipitation with iron also controlled by multicommutation.

2. Experimental

2.1. Apparatus and reagents

A PSA Millennium Merlin 10025 instrument from PS Analytical (Orpington, UK) was employed as mercury fluo-

rescence detector. The apparatus was equipped with two independent peristaltic pumps for the continuous fluorescence measurements; a Perma Pure[®] drier unit, a gas-liquid separation chamber, a photomultiplier tube and a data acquisition system (see Fig. 1 for the corresponding set-up).

The multicommutated flow system comprised four three-way solenoid valves (NResearch, 161T031), reaction coils on PTFE tubes of 0.8 mm i.d. and three T-type junctions mechanised in acrylic, which can be identified in Fig. 2.

Instrumental conditions employed for the CV-AFS determination of mercury in milk are indicated in Table 1. The multicommutated flow system was coupled to the PSA Millennium Merlin by connecting the valves before the gas-liquid separator. To control signals and to switch on/off the solenoid valves a microcomputer PCL-711 interface card (American Advantec Corp.) running with a designed software written in Quick BASIC 4.5 was used coupled with an external power supply interface to match the electric current intensity and voltage required by the three-way solenoid valves.

A domestic microwave oven LG Intellrowave (Manchester, UK) of 2450 MHz frequency and operating at a maximum exit power of 700 W was employed for microwave-assisted digestion of milk samples using a home made 115 ml internal volume PTFE reactor for the pressurized treatment.

Heavy metals control of the effluents was carried out by using an inductively coupled plasma mass spectrometer ICP-MS Perkin-Elmer Elan-5000 SCIEX (Thornhill, Ontario, Canada), working in the totalquant mode as indicated in a previous study [11].

All chemicals used were of the highest purity available and all solutions were prepared in nanopure water obtained

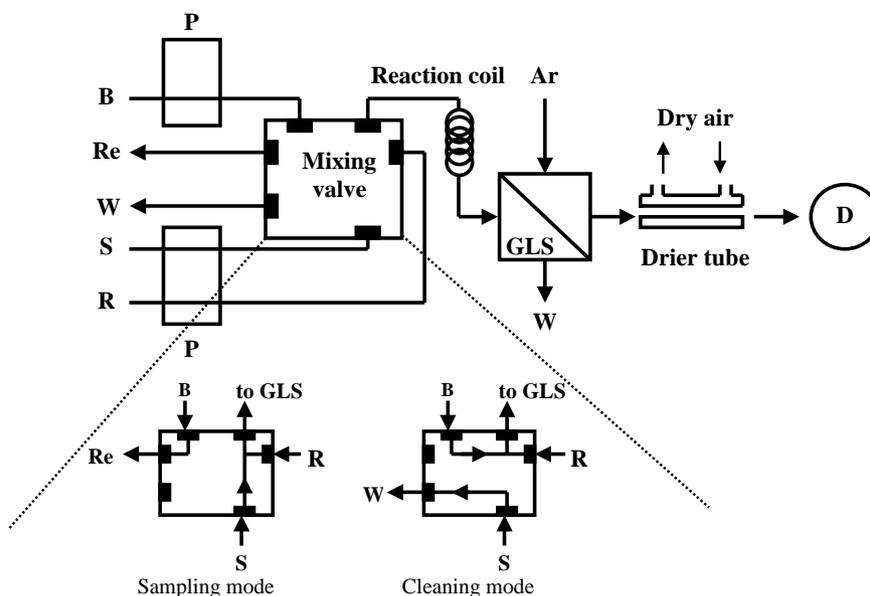


Fig. 1. Manifold employed for the continuous CV-AFS determination of Hg. R: reductant solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ with a flow rate of 4.1 ml min^{-1} ; S: standard or sample solutions at flow rate of 3.9 ml min^{-1} ; B: carrier blank solution at a flow rate of 4.5 ml min^{-1} ; Re: Recycling. GLS = 17 ml gas-liquid separator; D: detector; W: Waste; P: Peristaltic pump; Ar: argon carrier gas.

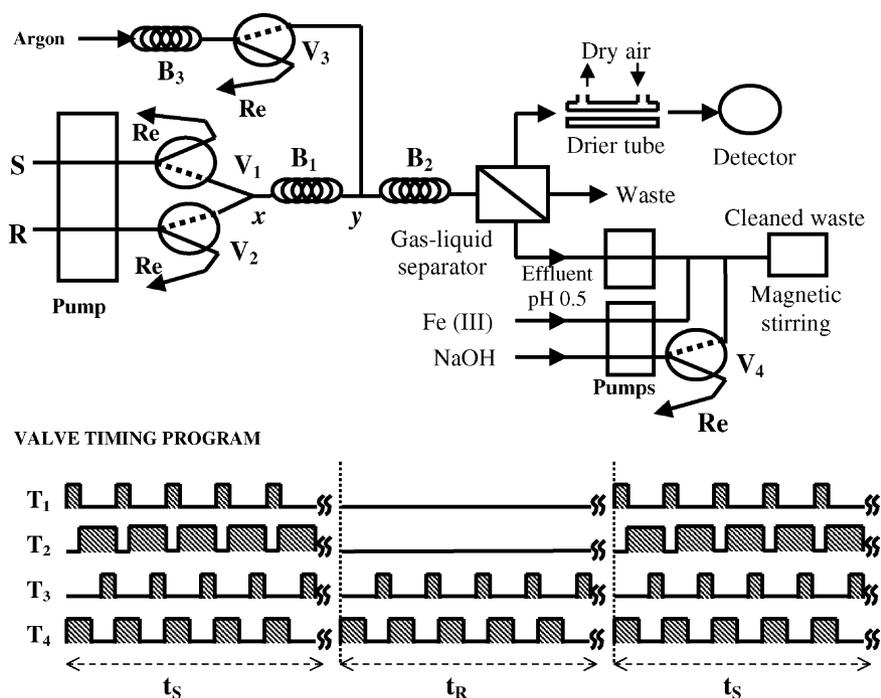


Fig. 2. Manifold employed for the multicommutation CV-AFS determination of Hg and valve timing program. R: reductant solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ at flow rate of 4.1 ml min^{-1} ; S: standard or sample solutions at flow rate of 3.9 ml min^{-1} ; Re: Recycling to the corresponding vessels; V_1 , V_2 , V_3 , V_4 : three-way solenoid valves; B_1 : reaction coil, 30 cm length, 0.8 mm i.d.; B_2 : gas diffusing coil, 47 cm length, 0.8 mm i.d.; B_3 : back pressure reducing coil, 250 cm length, 0.8 mm i.d.; x and y T type joint devices machined in acrylic; Fe(III) solutions of 40 mg l^{-1} at flow rate of 3.5 ml min^{-1} . NaOH 6 mol l^{-1} solution at 3.5 ml min^{-1} . (---) Valve switch on (bit 1 electronic position); (—) Valve switch off (bit 0 electronic position). In the timing program dark segments indicate switching on of valves; t_s : mixing time, t_r : reading time. T_1 , T_2 , T_3 and T_4 : times corresponding to V_1 , V_2 , V_3 and V_4 , respectively. Gas/liquid separator of 5 ml internal volume.

from a Milli-Q water purifier system Millipore (Bedford, USA), with a resistivity of $18.2 \text{ M}\Omega \text{ cm}$.

$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 10% (v/v) HCl was used as reductant agent, and prepared by dissolving the salt from Merck (Darmstadt, Germany) in 50% (v/v) HCl Scharlau (Barcelona, Spain), heating for 10 min and diluting with water. This solution was purged with nitrogen during 30 min in order to eliminate possible traces of mercury. A 10% (m/v) hydroxylamine hydrochloride was prepared by dissolving the salt from Merck in deionized water.

0.1 mol l^{-1} KBr and 0.02 mol l^{-1} KBrO_3 solution was prepared daily by dissolving KBr and KBrO_3 , both from Merck, in deionized water.

1000 mg l^{-1} Hg(II) standard stock solution was obtained from Merck. 100 ng ml^{-1} Hg(II) standard solution was prepared weekly and was used to prepare the corresponding calibration solutions from 0.05 to 1 ng ml^{-1} in 3 mol l^{-1} HCl, 8 mmol l^{-1} KBr and 1.6 mmol l^{-1} KBrO_3 and 1% (m/v) $\text{NH}_2\text{OH HCl}$ medium. The carrier blank solution was prepared by adding the appropriate amount of hydrochloric acid to reach 3 mol l^{-1} acid medium and the same amount of KBr/ KBrO_3 and hydroxylamine as employed for standard solutions.

40 mg l^{-1} Fe(III) solution was prepared by dissolving $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ from Probus (Barcelona, Spain) in deionized water. 6 mol l^{-1} NaOH was prepared by dissolving NaOH

from Probus in deionized water. These solutions were merged with the AFS waste stream and employed for waste minimization.

2.2. Flow system scheme and operating principles

Fig. 1 shows the manifold employed for the determination of Hg in milk by using the continuous AFS mode. As can be seen, sample or standard solutions were mixed with the $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ reductant reagent inside a reaction coil and the mixture passed to a 17 ml gas-liquid separation chamber. Mercury vapour obtained was transported to the detector through a drier tube by an argon flow. Alternatively, a carrier blank solution was merged with the reductant reagent in order to clean the system and to establish the baseline. Only blank solution was recycled and sample and reagents consumption depends on both, the time of operation and the flow rate values used.

The multicommutation set-up was developed to reach the as low as possible waste volume and reagents consume. Fig. 2 describes the multicommutated flow system based on the use of four three-way solenoid valves which is basically the same than that used previously for Hg determination in water samples [13]. In order to minimize argon consumption and to avoid sample dispersion, the gas stream was introduced into the analytical path. In the configuration

Table 1
Instrumental conditions employed for the determination of mercury in milk samples by CV-AFS

Parameter	Continuous mode	Multicommutation mode
Wavelength (nm)	354	
Measurement mode	Peak height	
HCl (mol l ⁻¹)	3	
SnCl ₂ % (m/v)	2.5	
Ar flow rate (ml min ⁻¹)	330	350
Air flow rate (l min ⁻¹)	2.5	2.5
Blank flow rate (ml min ⁻¹)	4.5	–
Sample flow rate (ml min ⁻¹)	3.9	3.9
SnCl ₂ flow rate (ml min ⁻¹)	4.1	4.1
Reaction coil length (cm)	100	–
Gas–liquid separator (ml)	17	5
B ₁ coil length (cm)	–	30
B ₂ coil length (cm)	–	47
B ₃ coil length (cm)	–	250
On/off sampling time (s) tv ₁ -on/tv ₁ -off	–	0.25/0.75
Sampling time cycles <i>n</i> ₁	–	20
On/off reagent time (s) tv ₂ -on/tv ₂ -off	–	0.75/0.25
Reagent time cycles <i>n</i> ₂	–	20
On/off argon time (s) tv ₃ -on/tv ₃ -off	–	0.25/0.75
Argon time cycles <i>n</i> ₃	–	During all sampling and reading times
Mixing time (s) tv ₁ <i>n</i> ₁ + tv ₂ <i>n</i> ₂ = <i>t</i> _s	60	20
Reading time (s)	52	31

showed valves V₁ (sample valve), V₂ (reductant valve) and V₃ (argon carrier valve) were switched off (solid line) at the beginning of the cycle. In this position, sample (S) and reagent (R) were pumped back to their reservoir vessels, and argon stream did not pass through the system. When switched on, Ar stream flowed through the coil B₂ towards the gas–liquid separation chamber and the detector. When the software was run, as can be seen in the valve timing program, the solenoid valves V₁ and V₂ were switched on/off alternatively (0.25/0.75 s and 0.75/0.25 s, respectively), thus sample and reductant solutions merge together through the confluence point *x* into the reaction coil B₁. Valve V₃ was switched on after 0.75 s and was switched on/off with a sequence of 0.25/0.75 s. After finishing the sampling step, which was defined by the mixing time (*t*_s), valves V₁ and V₂ were switched off and V₃ valve was maintained switched on/off all the time. Mixing between sample and reductant solutions take place during the transport through reaction coil B₁ and reduction of Hg(II) to elemental Hg occurred. This mode of operation is a little bit different than that employed before [12,13] and provided a strong reduction of sample and reagents consumption and waste generation.

While sample and reductant valves were switched on and off to perform the sampling steps in the mixing time (*t*_s), valve V₃ was switched on/off, 0.25/0.75 s respectively, several times, as can be seen in Table 1. Under these conditions,

the insertion of the argon stream into the analytical path was discontinuous. So, this design involves the use of a minimum flow rate of argon through coil B₂ and gas phase separation chamber. When the mixing time step was finished, V₃ were switch on/off during the reading time (*t*_R) to allow argon to pass to the reaction coil B₂ and transport both, the solution to the gas–liquid separator and Hg vapour to the detector.

All experiments were carried out in a home made gas–liquid separation chamber with an inner volume of 5 ml. The sample and reagent solutions flow rates were maintained at 3.9 and 4.1 ml min⁻¹, respectively, by using both, multicommutation and continuous measurement modes and an additional blank carrier flow of 4.5 ml min⁻¹ was used for the continuous mode. Argon stream flow rate used in multicommutation and continuous mode were 350 and 330 ml min⁻¹, as it has been indicated in Table 1.

Waste treatment was carried out on-line by introducing the V₄ three-way valve that controls a NaOH solution. The alternatively switching on/off of valve V₄ during 0.5/0.5 s, respectively was enough to neutralize the acid waste solution to a pH around 7.5. At this pH iron was precipitated as Fe(OH)₃ and heavy metals present in the liquid waste precipitated or coprecipitated with iron, thus providing a reduced volume of solid waste and a clear solution.

2.3. Experimental procedure

2.3.1. Sonication of samples with aqua regia

2.0000 ± 0.0001 g of liquid sample or 1.0000 ± 0.0001 g of milk powder were weighted and 2 ml of aqua regia were added. After shaking the mixture in an ultrasound water bath for 10 min, 0.5 ml of antifoam A and 2.5 ml 10% (m/v) hydroxylamine solution were added together with 6.25 ml conc. HCl in order to achieve an adequate medium for Hg determination. Finally, 2 ml of a 0.1 mol l⁻¹ KBr and 0.02 mol l⁻¹ KBrO₃ solution was added and diluted to 25 ml. The obtained sample slurry was introduced in both, the continuous and multicommutated, set-ups for Hg determination by CV-AFS.

2.3.2. Microwave-assisted digestion of the samples

2.0000 ± 0.0001 g of liquid sample were accurately weighted and 1.5 ml conc. HNO₃ were added. The mixture was placed in a PTFE reactor, which was closed and irradiated three times in cycles of 1 min at 500 W plus 10 min at 0 W. After digestion, the reactor was cooled inside an ice bath and 0.5 ml H₂O₂ 30% (m/v) and 0.5 ml conc. HNO₃ were added. The irradiation cycles were repeated till a clear solution was obtained. Finally, 0.25 g of hydroxylamine, 6.25 ml of conc. HCl and 2 ml of a 0.1 mol l⁻¹ KBr/0.02 mol l⁻¹ KBrO₃ solution were added and the sample was diluted to 25 ml with nanopure water. A beaker with 100 ml of deionized water was introduced inside the microwave oven, in order to prevent damage of the magnetron. Water was changed after every step.

For the analysis of milk powder 1.0000 ± 0.0001 g was weighted and reconstituted with 2 ml of water prior to digestion as it was described above. Mercury determination in digested samples was carried out by using both, continuous and multicommutation modes, following the indications employed for the use of the corresponding manifolds and interpolating the sample fluorescence measurements in external calibration lines obtained for solutions of Hg in the range from 0.05 to 1 ng ml^{-1} prepared with the same reagents addition than samples.

3. Results and discussion

3.1. Determination of Hg by using slurries of samples sonicated with aqua regia

In a previous work [14] they were established the best conditions for extraction of As, Sb, Se, Te and Bi from milk, being employed 2 ml aqua regia and sonication for 10 min for the treatment of 1 g of milk without phase separation, thus providing acidic slurries from which the corresponding hydrides could be generated and determined by AFS.

In mercury determination 1 ml aqua regia for the treatment of 1 g of milk was enough to obtain quantitative recoveries, but the use of 0.5 ml aqua regia and the same sonication time only produces a 60% recovery.

The role of hydroxylamine and KBr/KBrO₃ solution was to avoid a possible retention of mercury due to a reduction to the zero oxidation state before reaction with SnCl₂. In fact, when the aforementioned chemical agents were not added, the signal produced by a standard Hg solution was reduced up to a 20% after 1 h. The addition of in first place, hydroxylamine, and after that KBr and KBrO₃ assured the release of mercury during the measurement step and a stability of several hours for samples and standards.

3.2. Microwave digestion of milk samples

Previous experiments were carried out following a procedure used for As and Sb determination in milk [16], in which liquid samples were dried as safety measure in order to avoid overpressure inside the Teflon reactor. However it was found mercury losses during oven heating in open systems and because of that, liquid samples were treated directly in a closed reactor for Hg determination.

Based on our previous experience HNO₃ and HNO₃ plus H₂O₂ were assayed for sample digestion, also including fat treatment, using different volumes of reagents and irradiation times in order to find the best conditions.

It was found (see Table 2) that the use of H₂O₂ was necessary to obtain quantitative recoveries of Hg and that several short irradiation cycles of 1 min provided better recoveries than the use of a reduced number of cycles of 2 or 3 min. Additionally, the use of a mixture of HNO₃ and H₂O₂ in

Table 2

Effect of digestion conditions on the recovery of Hg after microwave-assisted digestion of 2.0000 ± 0.0001 g of full cream milk

Conditions	Hg recovery (%)
1 ml HNO ₃ , 3 cycles, 3 min	34 ± 5%
1 ml HNO ₃ , 2 cycles, 2 min + 1 ml HNO ₃ , 2 cycles, 2 min	61 ± 4%
1 ml HNO ₃ , 2 cycles, 1 min + 1 ml H ₂ O ₂ , 2 cycles, 1 min	83 ± 6%
1.5 ml HNO ₃ , 3 cycles, 1 min + 0.5 ml HNO ₃ and 0.5 ml H ₂ O ₂ , 3 cycles, 1 min	98 ± 5%

the last step provides a complete digestion of fat and assures safety conditions, which elongates average life-time of reactors.

3.3. Multiparametric study of the reductant and acid concentrations for the CV-AFS determination of Hg

In order to achieve the best experimental conditions for CV-AFS measurement of Hg, a series of experiments were designed by using sample slurries in both, continuous and multicommutated modes.

Reductant, carrier blank and sample flows, reaction coil length, gas–liquid separator volume and argon flow rate are experimental parameters which can not be modified easily with the available instrumentation and thus, fixed values indicated in Table 1 were employed for the optimisation of reductant and acid concentrations.

It was made a factorial design to study the effect on fluorescence signals of HCl concentration (in the range between 1 and 6 mol l^{-1}) and reductant concentration (in the range from 0.5 to 3.0% (m/v)). Calculations made using the SYSTAT 5.01 provided results indicated in Fig. 3a for continuous and Fig. 3b for multicommutation approaches.

It can be observed that in both cases, the maximum response corresponded to 3 mol l^{-1} HCl and 2.5% (m/v) SnCl₂. So these concentrations were chosen to carry out CV-AFS measurements.

Otherwise, ANOVA test demonstrated a strong correlation between the two parameters and fluorescence signal, being obtained a correlation coefficient of 0.98.

3.4. Analytical figures of merit

Figures of merit of CV-AFS determination of Hg are summarized in Table 3. Typical regression lines between peak height fluorescence measurements and Hg concentration were described by equations: $I_F = (1700 \pm 40)C + (80 \pm 50)$ for continuous and $I_F = (1645 \pm 15)C + (50 \pm 20)$ (C in ng ml^{-1}) for multicommutated modes, thus indicating that both sensitivity values were statistically comparables, and little differences could be attributed to random experimental factors. However, it must be noticed that when using multicommutation, a reduced volume of the gas–liquid separator was employed, therefore reducing the sample dis-

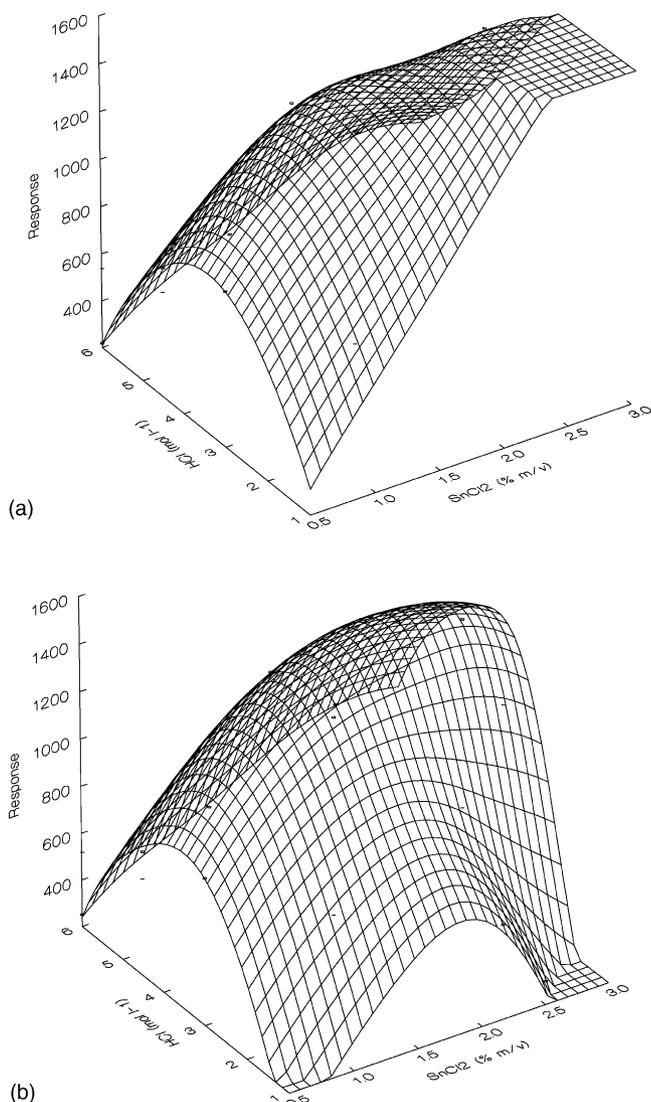


Fig. 3. Three-dimensional full factorial design results obtained for the study of the best experimental conditions for HCl and SnCl₂ concentrations on Hg determination by CV-AFS using continuous (a) and multicommutated (b) modes.

persion, which compensates the reduction of sample volume for 731–32.5 ml for each analysis.

Precision data obtained by multicommutation method was evaluated by measuring nine times the signal provided by a 0.05 ng ml⁻¹ standard solution of Hg(II), and as can be seen in Fig. 4 an excellent repeatability (R.S.D. 1.8%) was achieved, which compares well with that found by continuous measurement (R.S.D. 1.2%). The precision of the method was evaluated after five independent analysis of a sample containing 0.30 ng g⁻¹ and the relative standard deviation was found to be 3.4% in the multicommutation mode and 2.8% in the continuous mode. The small signals preceding the Hg analytical signals were due to valve swapping that produces a change of pressure in the flow system. However, this physical phenomenon does not affects the measurements, and it is hardly visible at Hg concentra-

Table 3
Analytical parameters of CV-AFS determination of Hg by using the continuous and multicommutation modes

	Continuous AFS	Multicommutation AFS
Calibration line	$I_F = (1700 \pm 40)C + (80 \pm 50)$	$I_F = (1645 \pm 15)C + (50 \pm 20)$
Correlation coefficient (r)	0.9985	0.9997
R.S.D. (%) ^a	1.2	1.8
R.S.D. (%) ^b	2.8	3.4
LOD (ng l ⁻¹) ^c	0.2	0.9
LOD (ng g ⁻¹) ^d	0.0025	0.011
Sample (ml) ^e	731 ^g	32.5
Reagent (ml) ^e	769 ^g	102.5
Carrier blank (ml) ^e	390 ^h	–
Ar consumption (ml min ⁻¹)	330	350
Waste (ml) ^f	605	94.5
Throughput (h ⁻¹)	32	70

^a R.S.D. (%): relative standard deviation corresponding to nine independent measurements of a solution containing 0.05 ng ml⁻¹ Hg.

^b R.S.D. (%): relative standard deviation corresponding to five independent analysis of a sample containing 0.30 ng g⁻¹ Hg.

^c LOD: limit of detection concerning diluted samples.

^d LOD: limit of detection regarding the original sample.

^e Sample and reagent consumption corresponding to 100 analysis.

^f Waste generated consumption was established for 1 h working session.

^g Values calculated for 112.5 s per analysis (mixing time (60 s) plus delay and memory time (52 s)).

^h Values calculated for 52 s (delay and memory time), because blank solution was recycling during mixing time in continuous mode.

tions higher than 0.1 ng ml⁻¹ (as it can be seen in the inset of Fig. 4).

The limit of detection obtained by CV-AFS varied from 0.2 to 0.9 ng l⁻¹ as a function of the use of continuous or multicommutated measurement modes, thus indicating the highest stability of blank measurements when the continuous mode was used. However, the 0.011 ng g⁻¹ LOD found for the original sample when the slurry approach was used in multicommutation CV-AFS clearly indicates that this methodology is suitable to obtain appropriate data for real samples.

Additional parameters which must be considered for method evaluation concern the sample and reagent consumption, waste generation and sample throughput, which affect the cost and speed of analysis. In these practical aspects it seems clear, from data in Table 3, that multicommutation provides a 22 times reduction of sample consume and 7.5 times reduction of reagent consumption, also avoiding the consumption of carrier blank.

On the other hand, the amount of waste generated, 94.5 ml for 1 h operating in the case of multicommutation as compared to 605 ml for continuous measurement involves an additional reduction of the cost of the analysis, specially when it is taken into consideration that by using multicommutation 70 samples can be measured in 1 h in front of 32 measured using the continuous mode.

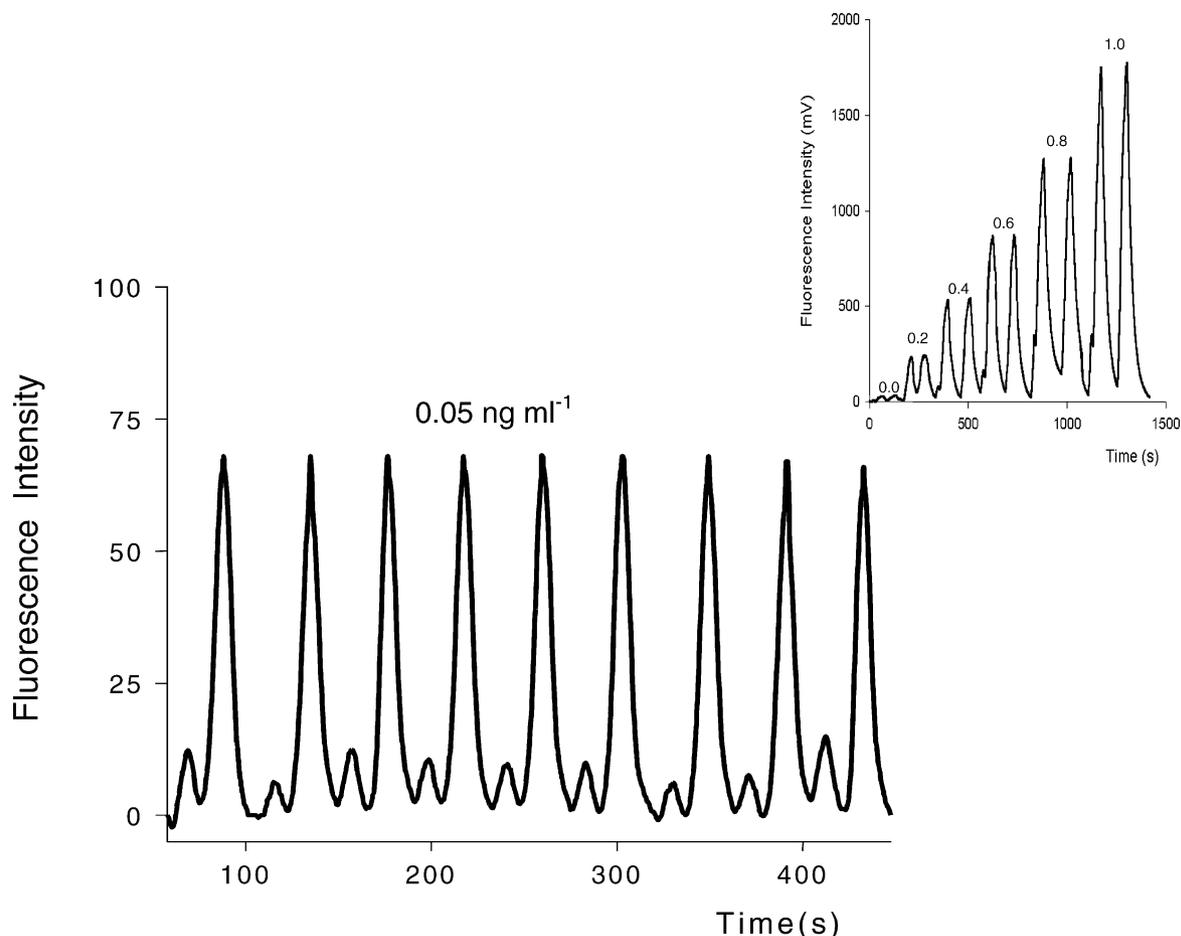


Fig. 4. Multicommutated fluorescence signals obtained for a 0.05 ng ml^{-1} Hg(II) standard solution. Inset: Fluorescence signals corresponding to two measurements of standard solutions of Hg from 0.0 to 1.0 ng ml^{-1} .

3.5. Mercury determination in market available milk samples

Data reported in Table 4 evidenced the presence of very low concentrations of Hg, from 0.09 ng g^{-1} (skimmed milk) to 0.61 ng g^{-1} (partially skimmed milk with propolis).

On considering the Hg content in the different samples assayed it can be concluded that the use of additives which

suppose an extraordinary fee of essential oligoelements and vitamins also could be responsible for a little bit increase of Hg concentration at trace levels. This fact is a general tendency observed for another elements like Se and Te [15] or As and Sb [16].

Additionally, it must be noticed that Hg was not detected in milk whey after protein precipitation with HCl, probably due to the retention of traces of Hg on coag-

Table 4

Total mercury concentration (ng g^{-1}) found in milk samples by slurries technique and microwave-assisted digestion

Milk	Slurries		Microwave digestion	
	Continuous	Multicommutation	Continuous	Multicommutation
Full cream cow milk	0.33 ± 0.03	0.29 ± 0.03	0.30 ± 0.01	0.31 ± 0.03
Semi-skim goat milk	0.48 ± 0.04	0.50 ± 0.06	0.52 ± 0.03	0.51 ± 0.07
Powder milk	0.25 ± 0.02	0.22 ± 0.05	0.24 ± 0.06	0.26 ± 0.04
Skimmed cow milk	0.10 ± 0.02	0.09 ± 0.04	0.12 ± 0.06	0.10 ± 0.05
Vegetable base cow milk	0.17 ± 0.02	0.16 ± 0.01	0.18 ± 0.01	0.19 ± 0.05
Partially skim cow milk with propolis	0.59 ± 0.03	0.61 ± 0.05	0.58 ± 0.02	0.60 ± 0.03
Full cream cow milk with Ca	0.21 ± 0.03	0.22 ± 0.03	0.23 ± 0.05	0.23 ± 0.07
Partially skim cow milk	0.22 ± 0.01	0.22 ± 0.04	0.18 ± 0.03	0.20 ± 0.06
NIST-1459	0.27 ± 0.06	0.2 ± 0.1	0.18 ± 0.05	0.21 ± 0.07

Measurements were carried out by continuous and multicommutation modes. Standard deviation corresponding to three independent analysis.

Table 5
Mercury recoveries (%) found in four commercially available milk samples by microwave digestion and slurries

Milk	Hg spiked	Slurries		Microwave digestion	
		Continuous	Multicommutation	Continuous	Multicommutation
Full cream cow milk	0.15	97 ± 2	96 ± 5	99 ± 3	95 ± 4
Semi-skim goat milk	0.2	93 ± 7	98 ± 3	94 ± 4	103 ± 2
Powder milk	0.25	101 ± 4	99 ± 3	104 ± 5	97 ± 6
Vegetable base cow milk	0.3	98 ± 3	102 ± 4	96 ± 4	94 ± 5

Measurements were carried out by continuous and multicommutation modes. Standard deviation corresponding to four independent replicates. *Note:* Hg spiked in ng g^{-1} added before treatment.

ulated proteins or because of the binding of this element.

3.6. Method validation

Recovery studies were made in order to evidence the lack of Hg losses or contamination during sample treatment and matrix interferences during the measurement step. Data found for different kind of samples (see Table 5) spiked with Hg concentrations ranging from 0.15 to 0.30 ng g^{-1} provided average recoveries of 98% for multicommutation CV-AFS determination of Hg in milk using slurries, thus evidencing the lack of systematic errors on using the developed procedure.

The comparison of data found for Hg by slurry sampling and multicommutation with those found by a microwave-assisted digestion provided an equation $y = (1.03 \pm 0.02)x - (0.01 \pm 0.01)$ with a regression coefficient of 0.99 and t calculated values for the slope (1.6) and for the intercept (1.8) which were in both cases lower than the theoretical t -value (2.04) for a 95% probability level and 30 freedom degrees thus showing a good comparability of the results, which evidences that the room-temperature sonication of samples is enough to obtain Hg concentrations of the same order than those obtained after sample digestion.

To validate the accuracy of the method a certified reference material NIST-1459 (non-fat milk powder) containing $0.3 \pm 0.2 \text{ ng g}^{-1}$ Hg was analysed and it was found a concentration of $0.27 \pm 0.06 \text{ ng g}^{-1}$. So indicating that the method developed is accurate.

3.7. On-line waste treatment

The new concept of Analytical Chemistry as a tool to control and solve problems implies the absence or minimal contamination side effects introduced by the analysis. In order to achieve this object it is desirable to treat the wastes on-line to avoid the stock of toxic residues.

The liquid effluent obtained from the CV-AFS system has a very acidic pH (0.5) due to the high quantity of HCl employed. So the merging of this waste, which has a flow of 8 ml min^{-1} , with a solution of Fe(III) at a flow of 3.5 ml min^{-1} followed by merging with a NaOH solution with a flow of 3.5 ml min^{-1} provides a good way for the on-line coprecipitation of heavy metals with $\text{Fe}(\text{OH})_3$.

The concentration of Fe(III) was varied from 10 to 50 mg l^{-1} and it was tested both the flow and the period of mixture with a solution of NaOH of different concentrations (from 2 to 6 mol l^{-1}) by coupling a three-way solenoid valve, V_4 , as can be seen in Fig. 2. This valve was switched on (discontinuous line)/off (solid line) alternatively at time of 0.5/0.5 s, respectively.

When valve V_4 was not running (off position), NaOH solution was pumped to its reservoir vessel. So, it was achieved a recirculation of sodium hydroxide and a minimisation of reagents consumption.

When a continuous flow of 40 mg l^{-1} of Fe(III) at a rate of 3.5 ml min^{-1} and an intermittent flow of 6 mol l^{-1} of NaOH were used, a neutral solution was obtained together with few grams of ferric hydroxide including the heavy metals present in samples and reagents. So, a drastic reduced of the amount of waste, from 1000 ml to 5 g for a working session was obtained, thus providing an environmentally friendly alternative to the use of the system in the absence of the deactivation treatment.

4. Conclusions

The developed procedure for mercury determination by CV-AFS using slurries and multicommutation offers a sensitive and accurate way for the determination of Hg in milk samples which avoids the laborious previous digestion of samples, being replaced this step by a single room-temperature sonication with aqua regia.

Multicommutated analysis mode allows us an easy mechanisation of the measurement step, a simplification of the experimental set-up, and the recirculation of samples and standards thus providing a minimal dilution of the samples and a drastic reduction of sample and reagents consumption.

Additionally, the use of multicommutation reduces the amount of liquid waste generation and improves the sample throughput. The on-line treatment of the liquid waste by coprecipitation with Fe(III) drastically reduces the amount of waste to a solid phase in which heavy metals are deactivated as compared with their mobility in the waste solutions. So, providing and on-line integration of both, the analysis and the waste decontamination [17].

On comparing the method proposed for Hg determination in milk with those previously developed by us for Hg in

water samples [12,13] it can be concluded that the discrete introduction of pulses of samples or standard solutions and reagents provides a reduction of sample volume consumption from 167 to 32.5 ml and a reagent volume consumption from 208 to 102.5 ml for 100 analysis, and also involves a reduction of waste volume from 193 ml to 94.5 ml h⁻¹. The detection limit was also improved from 1.3 to 0.9 ng l⁻¹ and the sample throughput from 49.5 to 70 analysis per hour, being the relative standard deviation value found 3.4%, higher than the previously obtained (0.1%), but taking into account the concentration of Hg in milk samples of the order of 0.30 ng g⁻¹, it could be considered highly satisfactory.

In conclusion, multicommutation system offers a sustainable and environmentally friendly alternative to minimise waste generations and then, the combination of multicommutation and on-line waste treatment offers the best alternative choice to the modern practical analytical procedures.

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